

The joint peristimulus–time scatter diagram is an index of the operational significance of a synapse

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A major goal in neurobiology is to trace the neural circuits responsible for simple behavioural acts, and to try to understand the properties of the behaviour in terms of those of the neural elements and the connexions between them. Once the outlines of a neural circuit have been obtained, the following question frequently arises: What is the functional significance of a particular connexion between neurones, or of a class of connexions among a population of neurones?

Statistical techniques have recently been developed to analyse the relations between the neural activity in two or more neurones^{2,3,10,11}. These methods, the joint peristimulus–time (PST) scatter diagram and cross-correlation histogram, are usually employed to deduce what connexions may be present or responsible for generating the observed relations^{1,5,8,9,13}. I report here a new experimental application of these techniques: the joint PST scatter diagram is used to test the effect and significance of known synaptic connexions.

The connexions treated in this report are the class of weak electrical synapses between tactile interneurons in the crayfish, *Procambarus clarkii*. The major input to these interneurons consists of chemically-mediated excitation from tactile afferents^{17,19}. When the interneurons are activated by tactile stimuli or by electric shocks to afferent nerves, the summated excitatory postsynaptic potential (EPSP) in an interneuron often includes small rapid unitary EPSPs which are generated by impulses in other interneurons. It has previously been demonstrated¹⁷ that these potentials are due to weak electrical connexions between some of the interneurons. These unitary EPSPs are always much less than the threshold for generating spikes. What, then, is the utility of these connexions, or are they merely artifacts of a loosely wired nervous system?

This question was approached by studying the response patterns of activity in two interneurons which were known to be coupled. I shall describe one typical experiment in detail. The dissection and stimulating and recording techniques are

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given elsewhere¹⁷. In the crayfish used for this experiment, intracellular recordings from an identified tactile interneurone (cell B or cell A63 of Wiersma and Hughes¹⁵) revealed a small electrical EPSP from a different tactile interneurone (cell C or cell A64 of Wiersma and Hughes¹⁵). Intracellular recordings from cell C failed to show any EPSP generated by cell B.

In a preliminary experiment, interneurone B was excited subliminally by tapping the laboratory bench, while interneurone C was dissected free from the nerve cord and stimulated directly. Under these conditions, the small coupling EPSP from interneurone C added to the 'background excitation' and succeeded in eliciting an impulse in interneurone B about one time in four. It seems, then, that the coupling between interneurons can be strong enough to lead to paired firing of interneurons in the usual context in which a crayfish finds itself, with some weak vibrations in the environment. Furthermore, afferent volleys elicited by shocks to a peripheral nerve or phasic mechanical stimulation of the carapace excited in the two interneurons discharges that appeared to be correlated (Fig. 1A).

In order to quantify these observations, the responses to 50 stimuli were grouped and the times of occurrence of impulses in the two cells were plotted as a joint PST scatter diagram^{2,3}, shown in Fig. 2A. The latency of each spike in interneurone C is

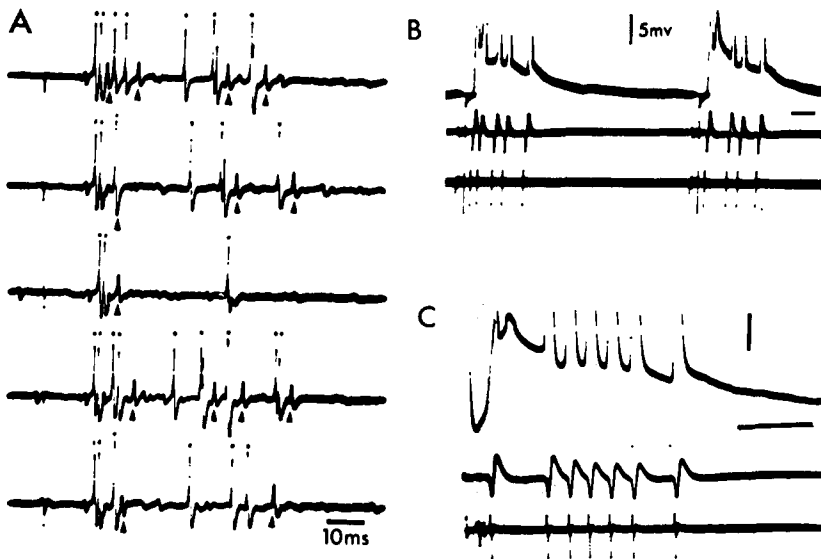


Fig. 1. A, Correlated firing in interneurons B and C in response to electrical shocks delivered to the second root of the third ganglion. The 5 traces show typical responses in tactile interneurons recorded in the nerve cord connective between the second and third ganglia. Spikes in interneurone B are indicated by a triangle; those of interneurone C are marked with a dot. Sections B and C of the figure show the basis for the pause in the discharge pattern of tactile interneurone C in response to root-two shocks applied in the third segment. Upper trace is intracellular recording from the third ganglion; voltage calibration applies to this trace only. Middle trace records activity in isolated axon of the interneurone in the 3/4 nerve cord connective. Bottom trace records activity of the same cell in the 2/3 connective. All spikes arise from the third ganglion. The recordings are selected responses to stimuli delivered at 10 Hz, using different sweep speeds.

paired with the latency to each spike in interneurone B to generate the abscissa and ordinate of each point. The presence of horizontal and vertical bands with few points indicates that both cells fire with an initial burst, followed by a pause, and then a second more dispersed burst of activity. The most interesting result comes from considering the activity in bands parallel to the 45° diagonal. Tallies in such bands are

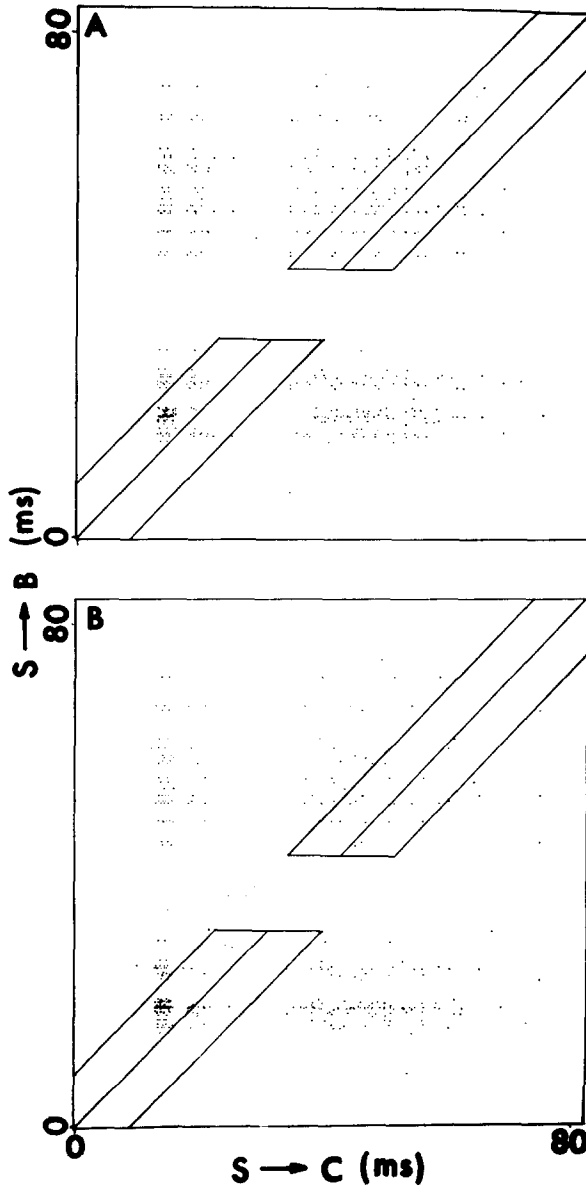


Fig. 2. Joint PST scatter diagrams for responses of interneurons B and C to orthodromic stimulation. Two groups of 25 stimuli delivered at 0.2 Hz are combined. Same experiment as Fig. 1A. Post-stimulus (S) latencies to spikes in interneurone B are plotted on the ordinate; latencies for interneurone C spikes are along the abscissa. Original data plotted in A; shuffled data in B (see text).

proportional to the cross-correlation histogram between the two neurones^{2,3}; that is, the distance of a point from the 45° diagonal is proportional to the difference between the times of occurrence of spikes in the two interneurons. It is evident from Fig. 2A that the density of points above the 45° diagonal is greater than the density below the diagonal. Thus, spikes in interneurone B tend to occur somewhat later than in interneurone C. This property of the cross-correlation histogram or the joint PST scatter diagram may indicate (1) an effective excitatory synaptic connexion from interneurone C to interneurone B. However, a similar result would occur if (2) the responses in interneurone B to the stimulus were on the average more delayed than the responses in interneurone C, if (3) the strength of the afferent volley, and hence the responses of the two interneurons, varied from stimulus to stimulus, or if (4) the two interneurons shared input from a third source independent of the stimulus, even if the neurones were not functionally connected.

The last possibility can be eliminated from information obtained in previously reported experiments. Analyses of the receptive fields of tactile interneurons^{6,15} as well as intracellular recordings^{6,17} show that the only excitatory inputs to tactile interneurons come from tactile afferents, or other tactile interneurons. Thus all inputs to the interneurons are silent in these experiments, except when activated by the stimulus.

The third possibility, variation in the strength of the afferent volley, was controlled by monitoring the amplitude and shape of the volley with a recording electrode proximal to the stimulating electrode. Supramaximal stimuli were applied to a branch of the second root of an abdominal ganglion. The afferent volley was constant. Using weaker stimuli, it could be shown that the loss of a single sensory neurone's contribution to the afferent volley could be detected.

In order to distinguish between the remaining two possible causes of the diagonal asymmetry of Fig. 1A, the response trains of interneurone B were shuffled with respect to the response trains of interneurone C and the stimulus, and a new joint PST scatter diagram was formed (Fig. 2B). The shuffling procedure matches the interneurone B responses following one particular stimulus to the responses of interneurone C following a randomly chosen stimulus. This destroys all significant time relationships between the two trains except those related directly to stimulus presentation. The resulting scatter diagram measures that part of the correlation between the interneurone trains due exclusively to shared activation by the stimulus, via the tactile afferents, regardless of direct interactions between cells B and C^{2,3,8}. Comparison of this control scatter diagram to the original derived from unshuffled data (Fig. 2A) permits distinction between diagonal components of the diagram due to common activation by the stimulus or to functional connexions between B and C.

The advantage of the joint PST scatter diagram over the cross-correlation histogram is that the former allows the correlation between two cells to be studied within different periods after the stimulus. Thus the joint PST scatter diagram introduces the additional dimension of the latency of a correlated response in two cells from the stimulus. The diagonal bands in Fig. 2 represent epochs of 8.35 msec about the null latency between spikes in the two interneurons. Two regions will be considered

separately: correlations between spikes occurring in the initial bursts of the two cells (the lower left cluster of points), and correlations between spikes occurring in the later firings of the cells (the upper right region of the diagrams).

Comparing the early bursts in the diagrams from the unshuffled and the shuffled data, little difference is seen. Whether shuffling was performed or not, spikes tend to occur later in interneurone B than in C, and the higher density above the diagonal cannot be attributed to any functional connexion between cells, for it would occur anyway to these stimulus volleys, due to the differences in response latencies. Specifically, in Fig. 2A, the lower band contains 9 points, and the upper band contains 84. In Fig. 2B, the two regions contain 14 and 80 points, respectively.

Consider, however, the correlation between the later spikes in the two cells seen in the upper right region of the diagrams. The original data has 34 points in the band above the diagonal, but only 13 points in the lower band. In the control figure, the upper band contains 9 points and the lower band has 10. The density of points in a sufficiently small region of a joint PST scatter diagram between independently firing neurones is approximately a Poisson random variable³. The 3 regions of low density reflecting no apparent correlations, having 9, 11 and 13 events, form an estimate of this variable, with both a mean and variance of 11. The probability that 34 is a member of the population described by this variable is negligible ($P < 0.01$, $t = 10.9$, $df = 2$). Using the observed sample variance and assuming Gaussian statistics, one obtains even more significance in the difference. Therefore, the observed apparent correlation between spikes in interneurone C and spikes occurring within 8.35 msec in interneurone B in the late part of the responses is real, and is destroyed by the procedure which preserves only correlations arising from shared activation by the stimulus. It may be concluded that the effect of the electrical synapse known to exist from interneurone C onto interneurone B in this animal is to cause the spikes in the cells to occur more synchronously than would happen otherwise. Similar analyses of the effect of known connexions between interneurones yielded qualitatively the same results in 4 crayfish.

Now that the effect of the coupling between tactile interneurones on their firing patterns has been determined, the question arises: What is the functional significance of the increased synchrony of discharges in the interneurones? The answer lies in knowing the efferent connexions of this population of cells. It is known that the tactile interneurones excite the lateral giant fibre electrically in each abdominal ganglion^{17,19}. This giant neurone is the command neurone or decision neurone responsible for eliciting single tail flips to phasic mechanical stimulation of the tail¹⁶. An impulse in this cell is a necessary and sufficient condition for the crayfish to generate this escape response to such stimuli. The lateral giant cell threshold, as seen from the synapses located on distal nonspiking dendrites, is very high, often exceeding 50 mV. This threshold is only reached when the tactile interneurones respond vigorously to a phasic tactile stimulus¹⁷. It is well known that a greater coherence or synchrony of spikes in a population of cells exciting a neurone will generate a larger compound EPSP in the follower neurone, and excite it more effectively¹². This, then, appears to be the ultimate result of the coupling between tactile interneurones: to increase the effectiveness of their activity in exciting a decision fibre for an escape behaviour.

The high threshold of the lateral giant makes the circuit very sensitive to changes in the density and coherence of spikes in the interneurons. This provision causes the lateral giant to fail to respond to stimuli which elicit weaker bursts in the interneurons as a consequence of depression in the afferent–interneuron chemical synapses. This arrangement results in an habituation of the response to repeated stimulation. The biophysical basis of habituation has been described in detail elsewhere¹⁸.

The experiments of Fig. 1 and 2 illustrate a frequently observed characteristic of the response patterns of tactile interneurons to phasic stimuli and afferent volleys^{6,7}. The discharges in any one interneuron often cluster into two groups, with a gap in between. The usual interpretation of such a result is that there is some inhibitory process which is expressed as the pause, or horizontal and vertical bands of low density in the joint PST scatter diagram^{2,10,11}. Intracellular recordings from interneurons displaying this pause contradict this interpretation, however (Fig. 1B and C). If the pause were due to some inhibitory process acting presynaptically or postsynaptically, one would expect to observe a transient reduction in the compound EPSP causing the spike discharges. No such reduction has been observed in over 30 recordings from tactile interneurons responding to an afferent volley to the ganglion of penetration. Rather, one frequently observes an abortive spike, either a local response⁴ or a branch spike^{6,7,14} which does not propagate into the axon. It appears, then, that the excitability of the axon of the interneuron is reduced during this brief period of strong depolarization, perhaps due to some sodium conductance inactivation or delayed rectification. Some evidence for such a short-term accommodation or relative refractoriness in these neurons has been reported¹⁴. When the afferent-generated depolarization to repetitive stimulation declines, the abortive spike may reach full size and invade the axon (Fig. 1B); then there is no pause in the firing pattern in the interneuron.

This example illustrates the difficulties and risks involved in trying to deduce the presence of postulated connexions onto neurons from an analysis of the form of their response patterns. Although it is for such purposes that these statistical techniques were developed, their limitations in the absence of relevant intracellular data pose serious problems in their application.

In contrast, I have used the joint PST scatter diagram of activity in coupled interneurons to show that the known synaptic connexions between the cells serve to group the discharges in the interneurons. A shuffling procedure was used to eliminate correlations arising from functional connexions, in order to distinguish them from correlations arising from shared excitation. It was also possible to use formal hypothesis testing to estimate the significance of the differences between the original and the control scatter diagrams. The success of the techniques in demonstrating that an observed connexion is really functional represents an important new type of conclusion about neural circuits that this type of analysis allows neurophysiologists to make.

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